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Research article

C–3 alkoxymethylation of 4-oxo-1,4-dihydroquinoline 2-carboxylic acid esters *via* organic additives

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ABSTRACT

Esters of kynurenic acid, a known neuroprotective agent were reacted with cyclic amino acids to yield novel alkoxymethylated products under optimized reaction conditions. The importance of amino acid based (primary, secondary, biogenic and synthetic) organic additives was proven by the conduction of numerous test reactions. Thoroughly extended investigations, directly focusing on amino acid catalysis, which is an emerging and up-to-date field of catalysis and green chemical processes, have been conducted. The mechanism of the alkoxymethylation reaction was proposed and later the findings supported the hypothesis of the first *retro*-Mannich step (formation of the *ortho*-quinone methide intermediate) and subsequent formation of the alkoxymethylated derivatives. As a preparative result, two novel kynurenic acid derivatives bearing an alkoxymethyl moiety and two additional derivatives having amino acid residues at the site C-3 were synthesized, thus setting the scope and limitations of the modified Mannich reaction of kynurenic acid derivatives using amino acid nucleophiles. The mechanistic investigations highlighted the significant physicochemical effects of used nucleophiles on the amino-acid driven one-pot *retro*-Mannich initiated alkoxylation of kynurenic acid.

1. Introduction

Kynurenic acid (KYNA) (**1**) is an endogenous quinoline derivative, biosynthesized from L-triptophane *via* the kynurenine pathway. The compound is of great importance due to its neuroprotective property as aphysiological levels are measured in a broad range of neurological disorders such as Parkinson and Huntington disease, epilepsy, and migraine [1–9].

Recent studies focused on the modification of kynurenic acid in order to enhance its penetrability through the blood–brain barrier. Amidation is a versatile means of structural fine tuning to form salts *via* substitution with diamines yielding compounds (**2a–b**) bearing tertiary amino moieties [10–12]. Another major method of structural fine tuning is the modified Mannich reaction on the analogy of 1-naphthol. In the presence of formaldehyde and a secondary amine, numerous C-3 aminoalkylated compounds were synthesized (**3a–c**) [12–16]. Compounds with both amide and aminoalkyl moieties were also successfully prepared (**4a–b**) (Fig. 1.) [11].

Amino acids are in focus regarding drug research due to their zwitterionic characteristics. Derivatives of widely known compounds, such as zidovudine [17], camptothecine [18], melphalan [19], and docetaxel [20] have been synthesized [21] (Fig. 2.)

The Mannich reaction is one of the known procedures to incorporate amino acid residues in compounds [22–26]. Performing the modified Mannich reaction, using kynurenic acid ethyl ester (**9**), paraformaldehyde, and L-proline (**10**) as an *N*-nucleophile, was

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attempted to synthesize a novel, zwitterionic kynurenic acid–amino acid hybrid (**11**).

2. Results

In our preliminary research, the reaction of compound **9** with paraformaldehyde and L-proline 1.0 equiv. each was conducted in absolute ethanol at 80 °C in a pressure-resistant vessel. The solvent was chosen on the basis of the ability of dissolving each reactant. After reaching a maximal conversion and then purification of the reaction mixture, the analysis of the product proved that the amino acid moiety is not present in the molecule. Instead, an unexpected product (**12**) bearing an ethoxymethylene moiety at the C-3 position was formed. Solvent ethanol acted as an *O*-nucleophile under the examined conditions, thus yielding an ether-type compound (Scheme 1.).

Product **12** attracted our attention, since it is a novel C-3 modified kynurenic acid derivative, able to broaden the spectrum of the possible neuroactive KYNA analogues.

In the literature a wide palette of alkoxyalkylation reactions of aromatic compounds bearing active hydrogen were published [27,28]. Reactions were carried out using catalysts such as boron- or phosphorous-modified HZSM-5 [29], ZBM-10 [30], HCl/HOAc/H_2O [31] or HCl/MeOH [32] systems or bis(trifluoromethanesulfonyl)imide [33]. Results of alkoxyalkylations *via* the usage of *N*-nucleophiles were disclosed starting with (−)-cannabidiol and (−)-cannabigerol [34] as well as resorcinarenes [35]. Iminodiacetic acid, an amino diacid was also applied as catalyst for alkoxyalkylation reactions of resorcinarene derivatives [36,37].

Having results of the preliminary reaction at hand, test reactions were conducted using common strong or weak acid or base additives. NaOEt, Et_3N, acetic acid or *p*-toluenesulfonic acid (1.0 equiv.) and 1.0 equiv. paraformaldehyde in abs. ethanol was added to **12**. Monitoring the reaction after 60 h indicated no conversion. Similar observation was made in microwave-assisted reactions at 100 and 120 °C temperatures. These findings led to the conclusion that for the formation of compound **12** a secondary *N*-nucleophile is necessary.

In the next step, **9** was reacted with 1.0 equiv. paraformaldehyde and 1.0 equiv. cyclic amines (**13**–**15**) in abs. ethanol in a pressure-resistant vessel for 60 h to reach maximum conversion [11]. Upon monitoring the reaction, crude NMR spectra and TLC-analysis showed that using pyrrolidine (**13**) and piperidine (**14**), no trace of **12** was observed. The only reaction products were aminoalkylated derivatives (**16, 17**). When morpholine (**15**), a significantly weaker base containing an *O*-heteroatom was used, ethoxymethylated compound **12** was detectable along with aminoalkylated product **18** (Scheme 2., Table 1.).

Based on this information, a series of reactions was conducted using acetate salts of **13**–**15** as *N*-nucleophiles (**19**–**21**). Compounds **19**–**21** are able to mimic the corresponding amino acids on the grounds of acidity as pyrrolidine acetate mimicked L-proline used previously. Compound **9** was reacted with 1.0 equiv. paraformaldehyde and acetates (**19**–**21**) in abs. ethanol until no change was detectable in conversion (Scheme 2.). Both crude NMR spectra and TLC-analysis showed that the conversion per cents of Mannich bases **16**–**18** dropped significantly in each reaction and compound **12** was detectable (Table 2.).

Full conversion per cents of **12** in the three test reactions were in the order of pyrrolidine acetate < piperidine acetate < morpholine acetate. These findings can be explained by either the electron-withdrawing ability of the oxygen atom in morpholine (thus making it the weakest base and strongest conjugate acid of the three compounds) or the presence of oxygen itself as a hydrogen-bond acceptor, able to coordinate an ethanol molecule.

Additional test reactions were run with L-proline (pK_a = 1.94, 10.33) (**10**), D-pipecolic acid (pK_a = 2.06, 10.39) (**22**), and (*S*)-morpholine-3-carboxylic acid (pK_a = 1.61, 8.52) (**23**) as *N*-nucleophiles. Compound **9** was reacted with 1.0 equiv. paraformaldehyde and amino acids (**10**, **22**, **23**) in abs. ethanol until no change in conversion was detectable. Having the crude NMR spectra at hand, it was found that C-3 aminoalkylated products could not be detected in either reaction (Scheme 3.).

In accordance with our previous results, maximal conversion per cents of **12** in the three test reactions were in the order of nucleophiles **10** < **22** < **23** used. These findings support the hypothesis of the importance of the oxygen heteroatom and prove that the acidic condition is necessary but it is not a sufficient factor regarding the formation of the ethoxymethylated product (Fig. 3.). The differences between conversions while using nucleophiles **10** or **19** and **20** or **22** can be explained with the steric effects regarding the ability of the nucleophilic attack between the 5-membered and 6-membered rings.

In order to clarify the previous results, eight more zwitterionic *N*-nucleophiles (**24**–**31**) were utilized in the test reactions (Table 3). Compound **9** was reacted with 1.0 equiv. paraformaldehyde and nucleophiles **24**–**31** in abs. ethanol in a pressure-resistant vessel for 60 h to reach maximum conversion.

Both crude NMR spectra and TLC-analysis showed no conversion of **9** in the case of nucleophiles **24**–**28**. Utilizing nucleophile **31**,

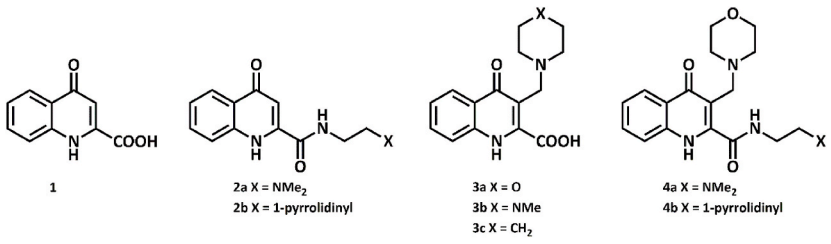


Fig. 1. Kynurenic acid and its chemically fine-tuned derivatives.

the maximal conversion per cent of **12** was found to be 15 %, which is significantly lower than that achieved with additive **23**. This information led to the deduction that only secondary *N*-nucleophiles facilitate the formation of compound **12**. Compound **31** being a potent acidic secondary amine nucleophile, the hypothesis is verified that for the ethoxymethylation reaction, an acid functional group on the nucleophile is necessary but not sufficient. Furthermore, the hydrogen-bond acceptor *O*-heteroatom is proposed to coordinate ethanol.

In the case of primary amino acid nucleophiles with side chains of negligible steric hindrance (**29**, **30**), stable Mannich bases **32**, **33** formed. It should be emphasized that in the case of compound **31** fair conversion and yield could be achieved (44 %), although **32** formed in a significantly lower conversion and yield (9 %) thus setting the scope and limitations of the original aim of the modified Mannich reaction of KYNA esters with amino acids (Scheme 4.).

In addition, we aimed to explore the mechanism of the ethoxymethylation reaction. It was hypothesized that first an unstable Mannich base forms (***I*** and zwitterionic ***I*-enol** and ***I*-oxo**) with the secondary amino acids. Next, upon the elimination of the amino acid, an *ortho*-quinone methide (***II***) forms which, *via* Michael addition, leads to compound **12** (Scheme 5.).

In order to elaborate the previous mechanism, esterification of compound **18** was conducted. Since utilizing thionyl chloride yielded complex reaction mixture, other coupling reagents (DIC, DCC, EDAC.HCl, CDI, HCTU, Ac_2O) were tested. Among these, *N*,*N*′-diisopropylcarbodiimide (DIC) in abs. ethanol at reflux temperature gave the highest conversion (Scheme 6.).

TLC and crude NMR analysis showed full converison of compound **18** (Fig. 4./A and B). Besides the formation of the aminoester (**34**), **12** also started to form as a side-product with approximately 1–7.7 M ratio (Fig. 4./B). Characteristic signals could be assigned to compound **34**. Note, however, that the signals of compound **12** are also detectable. The reaction was quenched and worked-up at that point. Surprisingly, after the work-up process and chromatographic purification, **12** was found to be present in significantly higher (2–3) molar ratio in comparison to **34** (Fig. 4./C) thus proving that compound **34** is highly prone to transformation to **12** also encumbering its isolation.

Formation of **12** indirectly proved the unstable amino acid Mannich ester (***I***) → *ortho*-quinone methide (***II***) → **12** route shown in Scheme 6. According to our proposed explanation, first the unisolable Mannich product ***I*** forms. Thanks to its zwitterionic trait, autoprotonation occurs yielding a quaternary ammonium compound at the benzylic site with good leaving group property. After the *retro*-Mannich type elimination of the amino acid, *ortho*-quinone methide (***II***), an excellent Michael acceptor forms, which readily reacts with the *O*-nucleophile solvent ethanol to form **12**.

As a next step the optimal equivalence of **23** was investigated in the reaction. Compound **23** was chosen for equivalence optimization as the highest conversion towards **12** could be achieved with this additive. In order to clarify the role of the catalyst, additive or reagent in the reaction of **23**, four test reactions were conducted with different equivalences. Compound **9** was reacted with paraformaldehyde and **23** (0.1, 0.5, 1.0, and 2.0 equiv.) in abs. ethanol until maximum conversions were observed. Crude NMR spectra of samples taken from the reaction chambers were analyzed and conversion curves were drawn (Fig. 5.).

The curves showed that a significant decrease in conversion occurred when applying 0.1 equiv. **23** in contrast to the utilization of 1.0 equivalent. When using 2.0 equiv. **23** the conversion of **12** measurably increased thanks to the higher molar ratio of the nucleophile. Interestingly, the optimal equivalent of **23** was found to be 0.5, an additive-range equivalent. Analyzing crude NMR spectra it can be hypothesized that lower nucleophile concentration subdues certain side reactions thus leading to higher conversion. These findings are supported by crude NMR-spectra since, in the case of the reaction with 0.5 equiv. **23**, less unidentifiable signals can be observed in contrast to using 1.0 or 2.0 equivalents of additive.

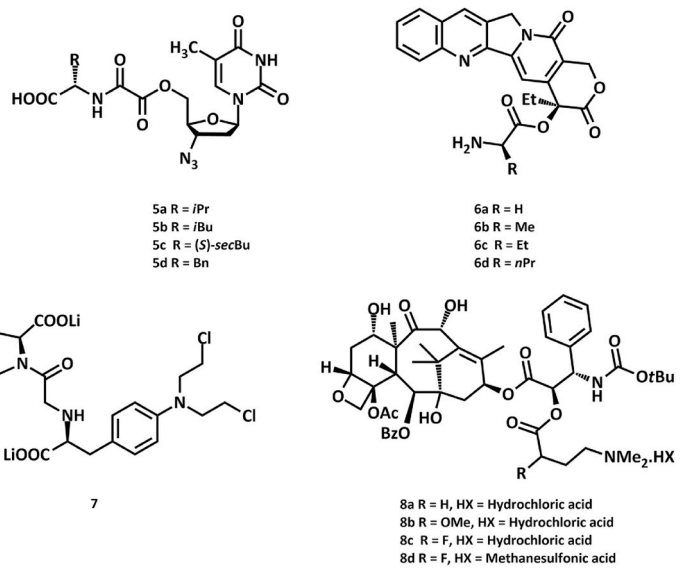
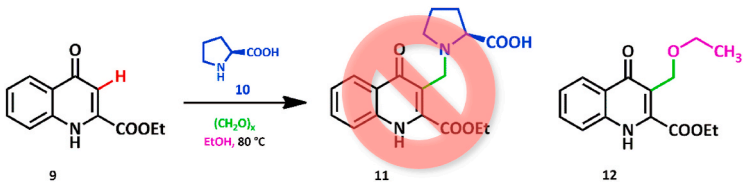
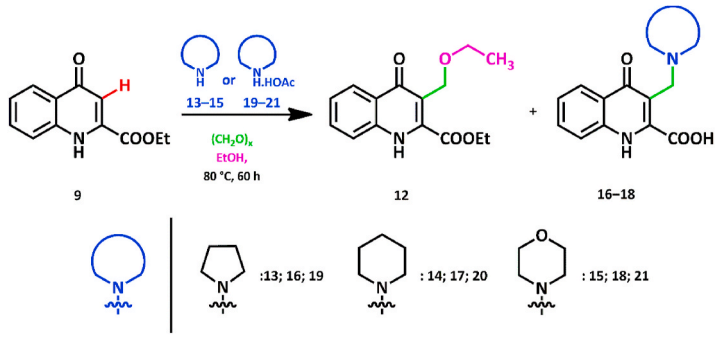


Fig. 2. Derivatives of zidovudine (**5a–d**), camptothecine (**6a–d**), melphalan (**7**), and docetaxel (**8a–d**) incorporating amino acid moieties.



Scheme 1. Modified Mannich reaction of **9** with L-proline and paraformaldehyde.



Scheme 2. Reaction of **9** with cyclic amines **13**–**15** or cyclic ammonium acetates **19**–**21** and paraformaldehyde in ethanol.

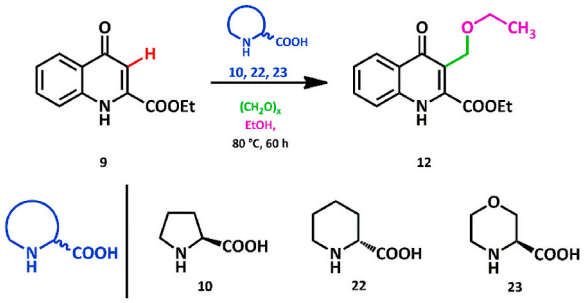
Table 1
Maximal conversion per cents measured in the reaction of **9** with cyclic amines **13**–**15** and paraformaldehyde in ethanol.

Used nucleophile		Maximal conversion		
#	pK_a	Mannich base		Compound 12
13	11.00	**16**	75 %[a]	–
14	11.00	**17**	98 %[a]	–
15	8.51	**18**	92 %[a]	4 %[b]

[a] Measured in 22.5-h reactions at plateau.
[b] Measured in 8-h reaction (**12** decomposed upon longer treatment).

Table 2
Maximal conversion per cents in the reaction of **9** with cyclic ammonium acetates **19**–**21** and paraformaldehyde in ethanol.

Used nucleophile			Maximal conversion (at reaction time)		
#	pK_a		Mannich bases		Compound 12
19	**HOAc**: 4.54	**13**: 11.00	**16**	56 % (28 h)	3 % (1–3 h)
20		**14**: 11.00	**17**	34 % (20 h)	5 % (1–5 h)
21		**15**: 8.51	**18**	60 % (12 h)	20 % (9 h)



Scheme 3. Reaction of **9** with cyclic amino acids **10**, **22**, **23** and paraformaldehyde in ethanol.

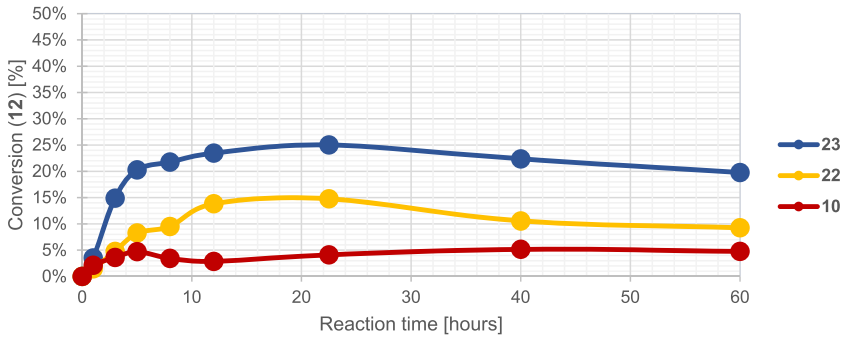
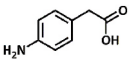
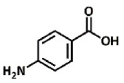
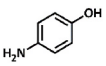
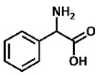
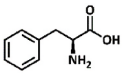
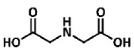
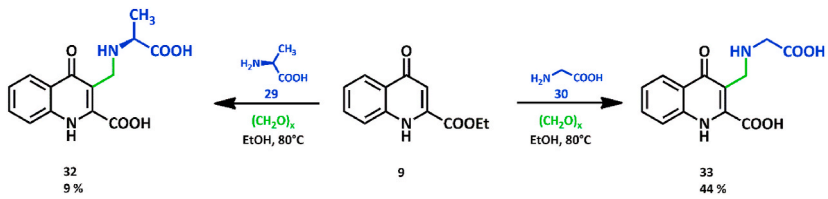


Fig. 3. Conversion curves of **12** depending of the used amino acid.

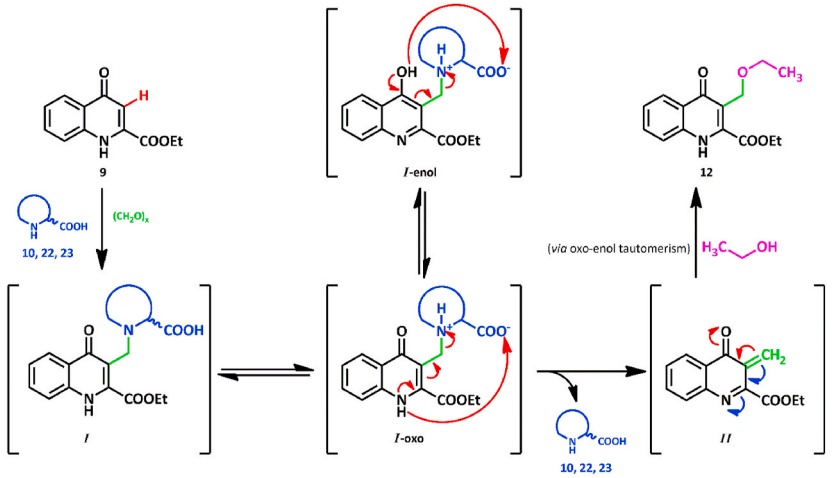
Table 3
Conversion per cents in the reactions of **9** with additional zwitterionic nucleophiles **24**–**31** and paraformaldehyde in ethanol.

Compound #	Structure	pK_a	Amine character	Acid character	Outcome
24		3.39, 5.51	Primary aromatic	Aliphatic	No conversion
25		2.69, 4.77	Primary aromatic	Aromatic	No conversion
26		5.43, 10.40	Primary aromatic	Weak phenolic	No conversion
27		2.29, 8.64	Primary aliphatic, at benzylic site	Aliphatic	No conversion
28		2.47, 9.45	Primary aliphatic	Aliphatic, large side-chain	No conversion
29		2.47, 9.48	Primary aliphatic	Aliphatic, short side-chain	Aminoalkylated product (**32**)
30		2.31, 9.24	Primary aliphatic	Aliphatic, no alkyl side-chain	Aminoalkylated product (**33**)
31		2.12, 2.90, 9.63	Secondary aliphatic	Aliphatic dicarboxylic acid	15 % conversion (**12**)

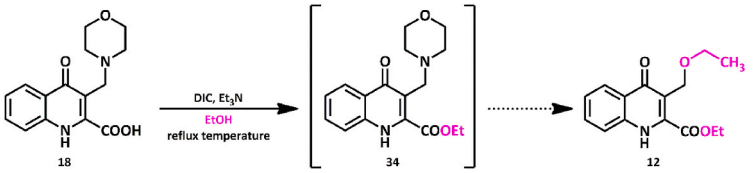


Scheme 4. Reaction of **9** with primary amino acids **29** and **30** and paraformaldehyde in ethanol.

Our next objective was to expand the scope of the reaction with diverse aldehyde or alcohol components. First, we changed the aldehyde. Compound **9** was reacted with 1.0 equiv. of the corresponding aldehyde (benzaldehyde, butyraldehyde, or phenylacetaldehyde) and 0.5 equiv. additive **23** in abs. ethanol. Both TLC and crude NMR analyses showed no conversion. A possible explanation of the failure of the reaction is the steric hindrance of the corresponding aldehydes [38]. Next, we modified the alcohol component in the reaction. We started with methanol the simplest alcohol followed by using bulkier primary, secondary, and tertiary alcohols. Kynurenic acid methyl ester [39] was reacted with 1.0 equiv. paraformaldehyde and 0.5 equiv. **23** in methanol for 60 h at



Scheme 5. Proposed mechanism of formation of **12** starting from compound **9**, using secondary amino acids **10, 22** or **34** and paraformaldehyde in ethanol.



Scheme 6. Esterification of Mannich base **18** with coupling agent DIC in ethanol.

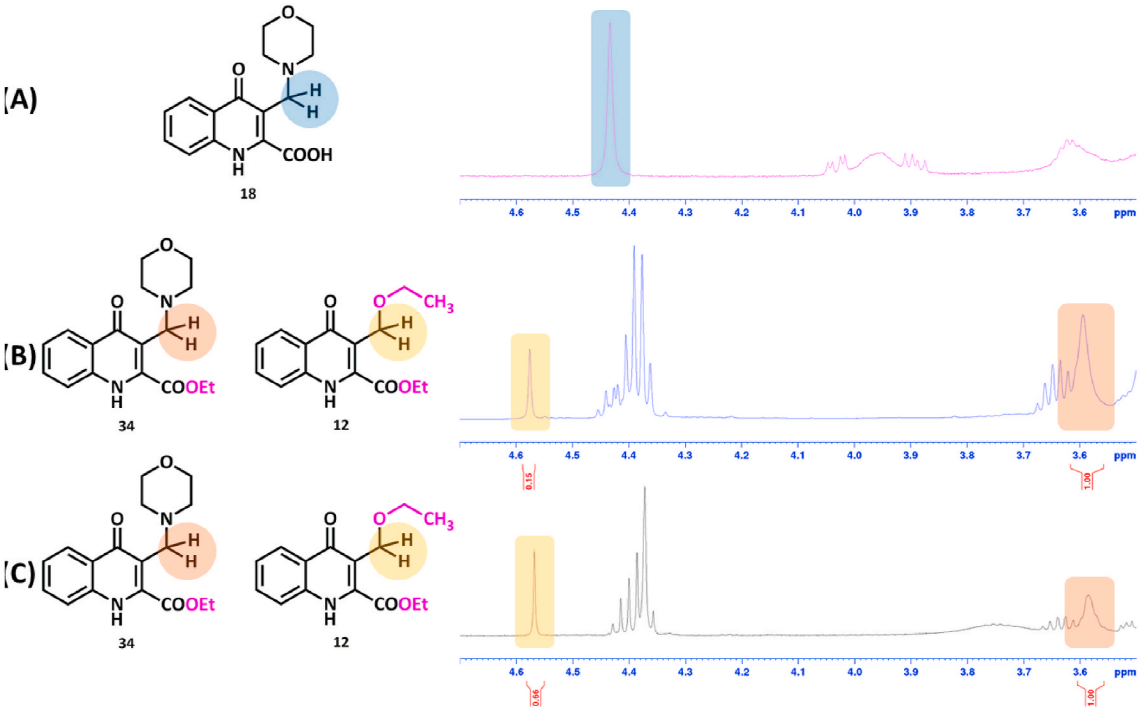


Fig. 4. Crude NMR spectra of the esterification reaction of **18** with coupling agent DIC in ethanol.

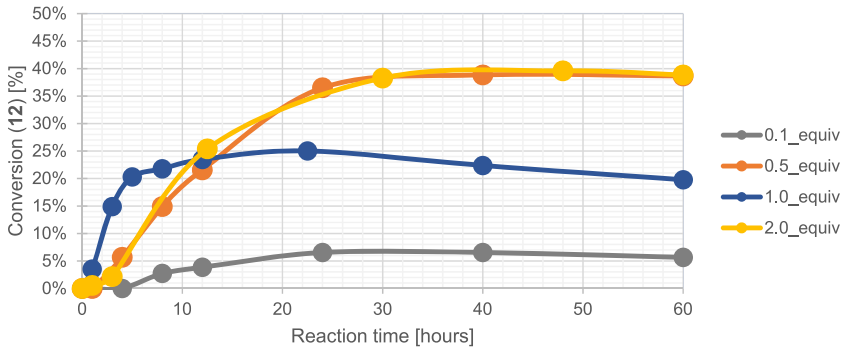


Fig. 5. Conversion curves (**12** assigned) of the C-3 ethoxymethylation reaction of **9** using different equivalents of **23**.

80 °C in a closed vessel. TLC and crude NMR analyses showed the formation of a complex reaction mixture from which the desired product was not possible to isolate.

Henceforth, compound **9** was reacted with 1.0 equiv. paraformaldehyde and 0.5 equiv. **23** in isopropyl alcohol for 60 h at 80 °C in a pressure-resistant vessel in order to produce the alkoxymethylated product. It was found that transesterification to the isopropyl ester occurred simultaneously to isopropoxymethylation. Only compound **35** formed (21 % maximal conversion in 26-h reaction) (Scheme 7.).

Conducting the reaction in *t*BuOH, a bulky tertiary alcohol, no conversion was observed after 60 h under the previously set conditions, thus setting the scope and limitations of the secondary aminoacid-mediated alkoxyalkylation reaction of kynurenic acid esters.

3. Materials and methods

For TLC analyses Merck Kieselgel 60 F_{254} plates were used. Melting points were measured on a Hinotek X-4 melting point apparatus with heating velocity of 4 °C/min. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 solutions in 5 mm tubes at room temperature (RT), on a Bruker DRX-500 spectrometer with a 5 mm BBO Prodigy Probe (Bruker Biospin, Karlsruhe, Baden Württemberg, Germany) at 500 (^{1}H) and 125 (^{13}C) MHz, with the deuterium signal of the solvent as the lock and TMS as internal standard (^{1}H, ^{13}C). Optical rotation values were measured on a Jasco P-2000 Polarimeter. The HRMS flow injection analysis was performed with Thermo Scientific Q Exactive Plus hybrid quadrupole-Orbitrap (Thermo Fisher Scientific, Waltham, MA, USA) mass spectrometer coupled to a Waters Acquity I-Class UPLC™ (Waters, Manchester, UK). FTIR spectra were measured on a Bruker Alpha II FTIR device in transmittance mode.

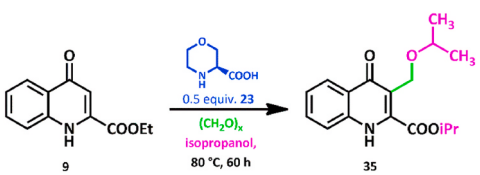
All chemicals were purchased from BLDPharm with the minimal purity of 95 %. The used solvents were absolute solvents. Chemicalize was used for pK_a calculations, 05.2024., https://chemicalize.com, developed by ChemAxon.

3.1. General procedure of the alkoxyalkylation and/or aminomethylation test reactions

4-Oxo-1,4-dihydroquinoline-2-carboxylic acid ethyl ester (**9**, 50 mg, 0.23 mmol) was dissolved in 3.0 mL absolute ethanol and then 7.0 mg paraformaldehyde (1.0 equivalent, 0.23 mmol), 1.0 equivalent (in case of **23** 0.1, 0.5, 1.0 or 2.0 equivalents) of the corresponding *N*-nucleophile (**10**, **13**–**15**, **19**–**31**) was added in a sealable tubular flask. The reaction chamber was sealed and heated in silicone oil bath at 80 °C for 60 h. Samples were taken at least four times. Before analysis, the solvent was evaporated followed by using the aforementioned NMR device.

3.1.1. Ethyl 3-(ethoxymethyl)-4-oxo-1,4-dihydroquinoline-2-carboxylate (12)

Synthesis was carried out according to the general procedure, then the solvent was evaporated and column chromatography was used (*n*-hexane: acetone 3 : 1) to purify compound **12** and then crystallized with *n*-hexane. Yield: 21 mg (33 %). M.p.: 98–102 °C. ^{1}H NMR (DMSO-d_6): 1.08 (t, 3H, $J = 7.15$ Hz); 1.39 (t, 3H, $J = 6.69$ Hz); 3.43 (q, 2H, $J = 7.21$ Hz); 4.42 (q, 2H, $J = 6.60$ Hz); 4.58 (s, 2H); 7.37 (t, 1H, $J = 6.70$ Hz); 7.70 (t, 1H, $J = 6.88$ Hz); 7.73 (d, 1H, $J = 7.95$ Hz); 8.12 (d, 1H, $J = 7.58$ Hz); 12.00 (brs, 1H). ^{13}C NMR



Scheme 7. Reaction of **9** with paraformaldehyde in isopropyl alcohol.

(DMSO-d_6): 14.25; 15.54; 62.25; 62.92; 65.49; 117.25; 119.30; 124.46; 124.96; 125.63; 132.94; 139.48; 140.16; 163.89; 176.58 (Figs. S1 and S2). HRMS calcd for $[M + H]^+$ m/z = 276.1230, found m/z = 276.1228 (Fig. S3). FTIR spectrum in Fig. S4.

3.1.2. Isopropyl 3-(isopropoxymethyl)-4-oxo-1,4-dihydroquinoline-2-carboxylate (**35**)

4-Oxo-1,4-dihydroquinoline-2-carboxylic acid ethyl ester (**9**, 50 mg, 0.23 mmol) was dissolved in 3.0 mL absolute isopropyl alcohol, and then 7.0 mg paraformaldehyde (1.0 equivalent, 0.23 mmol), 16 mg **23** (0.5 equivalent, 0.115 mmol) was added in a sealable tubular flask. The reaction chamber was sealed and heated in silicone oil bath at 80 °C. For the test reaction, 60-h reaction was carried out, whereas for the highest conversion and yield, 26-h reaction was used. Solvent was evaporated, followed by column chromatogprahy (*n*-hexane: acetone 3 : 1) to purify compound **35**, crystallized with *n*-hexane. Yield: 11 mg (16 %). M.p.: 142–144 °C. ^{1}H NMR (DMSO-d_6): 1.06 (d, 6H, J = 6.28 Hz); 1.39 (d, 6H, J = 6.28 Hz); 3.51–3.58 (m, 1H, J = 6.23 Hz); 4.57 (s, 2H); 5.15–5.24 (m, 1H, J = 6.53 Hz); 7.37 (t, 1H, J = 7.60 Hz); 7.70 (t, 1H, J = 6.93 Hz); 7.73 (d, 1H, J = 8.05 Hz); 8.10 (d, 1H, J = 8.58 Hz); 11.98 (brs, 1H). ^{13}C NMR (DMSO-d_6): 21.81; 22.47; 59.57; 70.55; 71.18; 117.47; 119.25; 124.40; 124.94; 125.65; 132.90; 139.46; 139.48; 155.00; 172.33 (Figs. S5 and S6). HRMS calcd for $[M + H]^+$ m/z = 304.1543, found m/z = 304.1540 (Fig. S7). FTIR spectrum in Fig. S8.

3.2. (S)-3-(((1-Carboxyethyl)amino)methyl)-4-oxo-1,4-dihydroquinoline-2-carboxylic acid (**32**)

4-Oxo-1,4-dihydroquinoline-2-carboxylic acid ethyl ester (**9**, 50 mg, 0.23 mmol) was dissolved in 3.0 mL ethanol and then 7 mg paraformaldehyde (1.0 equivalent, 0.23 mmol) and 1.0 equivalent of **29** (21 mg, 0.23 mmol) was added in a round-bottom flask. The reaction chamber was heated in silicone oil bath at reflux temperature for 95 h. Compound **32** crystallized from the reaction mixture. Crystals were filtered from the warm mixture and then washed with ethanol. Yield: 6 mg (9 %). M.p.: decomposes at 257 °C, $[\alpha]_D^{25}$ −26.45° (c 0.0128, H_2O). ^{1}H NMR (DMSO-d_6): 1.54 (d, 3H, J = 7.48 Hz) 4.35–4.45 (dd, 2H, J_1 = 17.96 Hz, J_2 = 25.73 Hz); 4.80 (q, 1H, J = 7.24 Hz); 7.40 (t, 1H, J = 7.57 Hz); 7.73 (t, 1H, J = 7.60 Hz); 7.81 (d, 1H, J = 8.39 Hz); 8.20 (d, 1H, J = 8.18 Hz). ^{13}C NMR (DMSO-d_6): 15.05; 44.27; 50.00; 119.32; 120.47; 121.77; 123.53; 124.87; 126.10; 132.18; 140.30; 163.82; 176.14; 178.16 (Figs. S9 and S10). HRMS calcd for of $[M - H_2O - H]^-$ m/z = 271.0727, found m/z = 271.0729 (Fig. S11). FTIR spectrum in Fig. S12.

3.3. (((Carboxymethyl)amino)methyl)-4-oxo-1,4-dihydroquinoline-2-carboxylic acid (**33**)

4-Oxo-1,4-dihydroquinoline-2-carboxylic acid ethyl ester (**9**, 50 mg, 0.23 mmol) was dissolved in 3.0 mL ethanol and then 7 mg paraformaldehyde (1.0 equivalent, 0.23 mmol), and 1.0 equivalent of **30** (18 mg, 0.23 mmol) was added in a round-bottom flask. The reaction chamber was heated in silicone oil bath at reflux temperature for 88 h. Compound **33** crystallized from the reaction mixture. Crystals filtered from the warm mixture were washed with ethanol. Yield: 28 mg (44 %). M.p.: decomposes at 231 °C. ^{1}H NMR (DMSO-d_6): 4.32 (s, 2H); 4.40 (s, 2H); 7.40 (t, 1H, J = 7.50 Hz); 7.73 (t, 1H, J = 7.77 Hz); 7.81 (d, 1H, J = 8.70 Hz); 8.20 (d, 1H, J = 8.04 Hz). ^{13}C NMR (DMSO-d_6): 43.99; 47.46; 119.33; 120.42; 123.55; 124.86; 126.13; 128.14; 132.20; 140.27; 163.95; 169.82; 173.75 (Figs. S13 and S14). HRMS calcd for $[M - H_2O - H]^-$ m/z = 257.0568, found m/z = 257.0569 (Fig. S15). FTIR spectrum in Fig. S16.

4. Conclusions

Novel C-3 alkoxymethylated derivatives of kynurenic acid were synthesized with cyclic secondary alpha amino acid additives. The importance of a secondary acidic *N*-nucleophile was proven. Test reactions were conducted using common organic additives, including cyclic amines and their acetate salts, as well as using other acidic *N*-nucleophiles. Result showed the importance of the local acidic conditions, particularly when using amino acids. Moreover, the importance of the corresponding heterocycle and its ability to coordinate the *O*-nucleophile were also highlighted. A mechanism of the alkoxyalkylation of kynurenic acid ethyl esters was proposed. Optimal equivalence of the amino acid was set. Two novel kynurenic acid derivatives, containing glycine or L-alanine fragments were synthesized, thus proving the scope and limitations of the aminoalkylation reaction of kynurenic acid ethyl ester using alpha amino acids. Limitations were set of alkoxyalkylation reactions and two novel kynurenic acid derivatives were synthesized bearing ethoxymethyl or isopropoxymethyl moieties at the site C-3.

CRediT authorship contribution statement

Péter Simon: Writing – review & editing, Writing – original draft, Investigation. **Bálint Lőrinczi:** Writing – review & editing, Writing – original draft, Investigation. **István Szatmári:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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Abbreviations

CDI	1,1′-Carbonyldiimidazole
DCC	*N*,*N*′-Dicyclohexylcarbodiimide
DIC	*N*,*N*′-Diisopropylcarbodiimide
DMSO-d_6	Deuterated dimethylsulfoxide
EDAC.HCl	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
HCTU	*O*-(1*H*-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HZSM-5	protonated form of Zeolite Socony Mobil-5
KYNA	Kynurenic acid
NMR	Nuclear Magnetic Resonance (spectroscopy or spectrum)
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
ZBM	Zeolite-bentonite Mixture(s)

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